Thesis of dissertation

ENVIRONMENTAL EFFECTS, PHYSIOLOGICAL STATE AND HOST-PARASITE ASSEMBLAGES IN EUROPEAN LIZARD SPECIES

Boglárka Mészáros

Department of Systematic Zoology and Ecology

Eötvös Loránd University

Supervisor:

Dr. János Török

professor

Doctorate School of Biology, ELTE

Head: **Dr. Anna Erdei**

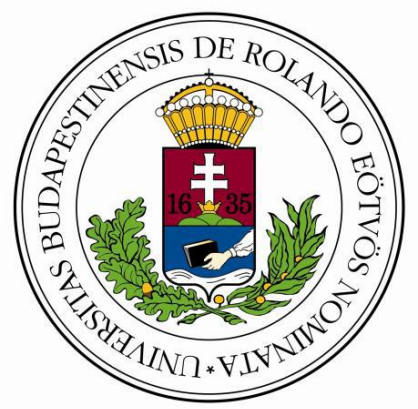
professor

Zootaxonomy, Ecology and Hydrobiology Ph.D. program

Program leader:

Dr. János Török

professor



BUDAPEST, 2020

1. Introduction

Evolution of sexual dimorphism and dichromatism was explained by the theory of sexual selection, first described by Charles Darwin in 1871. Sexual selection is the process of mate choice and intrasexual competition, during which individuals maximize their fitness by increasing their reproductive success (Andersson 1994). Sexual selection can manifest in the development of conspicuous signals (Darwin 1871). In reptiles, colouration can function as sexual signals providing honest information about several aspects of the bearer's health and physiological state during intra- and intersexual selection (Bajer *et al.* 2010, 2011, Hamilton *et al.* 2013, Names *et al.* 2019). Environmental factors (e.g. temperature, food availability, parasite infection) can have significant effects on the physiological state of ectotherms and thus can affect the expression of sexually selected colour signals (Huey és Kingsolver 1989, Pike *et al.* 2007, Bajer *et al.* 2012), however the available information on the underlying processes of these relationships is scarce. For instance, oxidative stress (Fang *és mtsai* 2002, Metcalfe és Alonso-Alvarez 2010) and immune efficiency (Polo-Cavia *és mtsai* 2013, Ibanez *és mtsai* 2014, Martín és López 2009) have been proposed to have important roles in internalizing and transferring the effects of environmental factors and determining the intensity of sexual signals. A different approach is to characterize the general physiological state of an individual via its locomotor performance, a highly temperature dependent attribute in ectotherms that shows many aspects of physiology and behaviour (Angilletta *és mtsai* 2002). As a biotic factor, parasite infection can also be a significant stressor. Theory of parasite mediated sexual selection argues that parasite infection can act as a basis for signal honesty, affect the intensity of sexual signals and may also affect signal evolution as an important selective force. The interspecific prediction of the theory says that species with higher parasite prevalence develop more intense and conspicuous signals because of the higher selective pressure. In contrast, parasite infection has lower selective force on signal intensity and results in less pronounced sexual signals in species with lower parasite prevalence (Hamilton és Zuk 1982). There are very few studies testing the theory and most of their results are contradictory (Cooper 2001), while in reptiles the interspecific prediction is not supported (Lefcort és Blaustein 1991, Schall és Staats 1997).

2. Aims

My doctoral research was carried out on Hungarian and Spanish lizard species, all of which belong to the family Lacertidae. Each of the examined species (*Lacerta viridis, Podarcis tauricus, Iberolacerta cyreni, Psammodromus algirus, Timon lepidus, Podarcis muralis, Podarcis guadarramae*) have conspicuous pigment-based and/or structural colouration that

presumably play important roles in communication and sexual selection. Because of these attributes the examined species prove to be an excellent model system for my doctoral research. The main aim of my work was to gain a better understanding of the environmental factors influencing colour signals and the possible underlying mechanisms. In order to do so, I have sought to answer the following questions:

I. Effects of environmental stress on physiological state and colouration: how does food availability and thermoregulation time influence the colouration of lizards and in parallel how does it affect oxidative state, immunocompetence and haematocrit level?
II. Effects of habitat quality on oxidative state and colouration: in habitats with different thermal qualities, what aspects of oxidative state relate to colouration and other traits of individual quality?
III. Relationship of food availability, locomotor performance, parasite infection and colouration: how does food deprivation affect the locomotor performance of lizards? How does parasite infection and colour intensity correlate with locomotor performance?
IV. Effects of parasite infection on colouration: how does parasite prevalence influence the expression of sexual signals in the examined lizard species?

3. Effects of environmental stress on physiological state and colouration

Materials and methods

I collected 40 adult European green lizards (*L. viridis*) to study the effects of food availability and thermoregulation time on physiological state and colouration. I conducted a 14 days long experiment where food availability and thermoregulation time was manipulated in a factorial design. I measured the morphological traits, the colour intensity of throat and belly, the oxidative state, the immunocompetence and the haematocrit of every individual before and after the experiment. A multivariate general linear model was built to study the effects of treatments on the changes of physiological and colour variables.

Results and discussion

The results of the multivariate general linear model showed that both treatments had significant effect on physiology and colouration. High food availability decreased immunocompetence while increased the amount of reactive oxygen metabolites and the brightness of throat colouration. These results suggest that individuals with higher food availability can allocate more energy into maintaining their colour signals, but higher metabolic

rate may increase the production of free radicals that damage the organism's cells and molecules and thus decrease immunocompetence. Besides this, individuals able to maintain their optimal body temperature for longer times showed stronger immune reaction but higher amounts of reactive oxygen metabolites than lizards with shorter thermoregulation time. Higher metabolic rate paired with higher oxidative damage but caused stronger immune reaction, indicating that developing an intense immune reaction is costly for the organism. In conclusion the honesty of the sexual signal of lizards is provided by a trade-off between signal intensity and physiology, and oxidative damage and immunocompetence could be important underlying mechanisms to transfer the effects of food availability and thermoregulation.

4. Effects of habitat quality on oxidative state and colouration

Materials and methods

In order to compare the oxidative state and individual quality of two ectotherm species living in different thermal habitats (stochastic and predictable), I collected 15-15 individuals of the Spanish *I. cyreni* and *P. algirus*. Individual quality was estimated by the relative intensity of throat, belly and ventrolateral colouration and the intensity of endo- and ectoparasite infection. To test my prediction I studied two different aspects of oxidative state: the amount of reactive oxygen metabolites produced at maximum locomotor performance (maximum ROM) and the temperature range where the reactive oxygen metabolit level is at least 80% of the maximum ROM level (effective ROM range). Relationship between individual quality and aspects of oxidative state were analysed by general/generalized linear models.

Results and discussion

Variables of oxidative state showed significant correlation with traits of individual quality in both species. I found negative correlation between the effective ROM range and the relative intensity of ventrolateral UV colouration in *I. cyreni*, which means that individuals that suffer higher oxidative damage in a wider temperature range have less intensive UV colouration. In contrast, I found a negative relationship between maximum ROM and the relative yellow intensity of throat colouration, so animals which show higher oxidative damage have less intensive throat colouration. The relative UV intensity of the belly also showed negative correlation with the maximum ROM. It suggests that individuals with low oxidative damage can only maintain intense structural colouration. Besides these, ectoparasite intensity positively correlated with maximum ROM which means that more intensely parasitized individuals suffered higher oxidative damage. The main finding of my study is that the sexual signals and

individual quality traits of species in different thermal habitats are affected by different aspects of oxidative damage. *P. algirus* lives in a more predictable thermal habitat. In this case the intensity of the sexual signal is determined by the level of maximum oxidative damage while in a more stochastic thermal habitat (*I. cyreni*) the width of the effective ROM range was decisive. In conclusion, adaptation to different environmental conditions may be expressed through different aspects of oxidative state.

5. Relationship of food availability, locomotor performance, parasite infection and colouration

Materials and methods

In order to study the effects of food deprivation on locomotor performance I collected 36-36 male European green lizards (*L. viridis*) and sand lizards (*P. tauricus*). After measuring morphology, colouration, ecto- and endoparasite infection intensity I divided the animals into two groups (control and deprived) and conducted a ten day long food treatment on both species. After the treatment I measured the thermal performance curves of each individual and then calculated the body temperature of maximum performance, breadth (effective performance), preferred body temperature of each individual and the performance on the preferred temperature. I used general linear models to analyse the effects of the food treatment and the correlations between the intensity of pigment-based colouration, UV colouration, parasite infection and locomotor performance.

Results and discussion

In European green lizards, food deprivation resulted in a wider effective performance range (breadth) which means that starving individuals could maintain the 80% of their maximal performance in a wider temperature range while lizards under optimal food treatment had a narrower performance breadth. It suggests that lizards under suboptimal food treatment could not reach their maximal performance which caused a flatter thermal performance curve and thus resulted in a wider breadth. Individuals have to compensate for the lack of available food. They are forced to establish a minimum level of performance which allows them to maintain a significantly lower but still sufficient performance below and above their optimal temperature. Beside this, the interaction of parasite intensity and food availability had a significant effect: after optimal food treatment, the more parasitized males reached their maximal performance on a higher temperature, while in the suboptimal food treatment group there was no interaction. This result suggests that more parasitized individuals are forced to increase their metabolic rate

in order to develop a higher immune response, which can be reached on higher body temperatures. Optimal nutrition enabled them to increase their metabolic rate while suboptimal food availability made it impossible. These findings suggest that thermal performance curves have a high plasticity and allows ectotherm organisms to adapt to different environmental factors. This plasticity may even enable them to reduce the negative effects of climate change.

In the sand lizard I found that males with more intense yellow throat colouration had wider performance breadth and decreased maximum performance. It is possible that maintaining an intense throat colouration requires more energy and thus decreases the energy available for allocation into locomotor performance. This trade-off between locomotor performance and pigment-based colouration provides the honesty of the sexual signal of male sand lizards. Besides these, I found that individuals with more intense ventrolateral UV colouration showed their maximum performance on higher temperatures. Structural colouration is energetically costly for the individual which can be provided by high metabolic rate and higher body temperature. This results in individuals with more intense structural colouration having a higher risk of overheating. Only individuals with better quality can afford these costs which suggests that the UV components of the ventrolateral blue spots may function as an honest sexually selected signal.

6. Effects of parasite infection on colouration

Materials and methods

To study the effects of parasite prevalence on sexual colour signals I collected male individuals of five Spanish lizard species (15 *I. cyreni*, 14 *P. algirus*, 11 *P. muralis*, 8 *P. guadarramae*, 5 *T. lepidus*). Morphological traits and relative intensity of pigment-based and structural colouration (throat, belly, ventrolateral spots) were measured on all individuals. I collected blood samples to estimate parasite intensity and prevalence and built multivariate general linear models to analyse the relationship between colouration and parasite prevalence.

Results and discussion

Parasite prevalence and snout-vent length had significant effects on the structural and pigment-based colouration. Prevalence affected the relative intensity of structural colouration of the throat, belly and ventrolateral spots while in case of pigment-based colouration prevalence was correlated with the relative intensity of throat and ventrolateral colouration. My results showed that both structural and pigment-based colour signals could be related to prevalence. In addition, the different colour traits correlated negatively, positively or did not

correlate with health state which suggests that these colour traits are the components of a multiple signalling system and are used to signal different aspects of individual quality. This preliminary examination suggests that parasite prevalence may have significant effects on the intensity of colour signals. In addition, it has showed that these lizard species could be an excellent model in the future to test the interspecific prediction of the Hamilton–Zuk hypothesis.

7. Publications, manuscripts and conferences included in the dissertation

Mészáros, B., Jordán, L., Bajer, K., Martín, J., Török, J., Molnár, O. (2019) Relationship between oxidative stress and sexual coloration of lizards depends on thermal habitat. *The Science of Nature*. 106: 9-10 p. 55. doi:10.1007/s00114-019-1649-2 (IF: 1,825)

Mészáros, B., Herczeg, G., Bajer, K., Török, J., Molnár, O. (2018) Effects of energy and thermoregulation time on physiological state and sexual signal in a lizard. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology.* 327 (9): 570-578. doi: 10.1002/jez.2143 (IF: 1,081)

Mészáros, B., Molnár, O., Bajer, K., Jordán, L., Martín, J., Török, J., (2019) Blood parasite infection as a possible selection force in the evolution of color signals in male Lacertid lizards. (*manuscript*)

Mészáros, B., Herczeg, G., Bajer, K., Török, J., Molnár, O. Környezeti stressz hatása a szexuális szignálokra és egészségi állapotra: kísérletes vizsgálat zöld gyíkon (*Lacerta viridis*). poster: *11. Magyar Ökológus Kongresszus* (28 – 30 August 2018, Nyíregyháza, Hungary)

Jordán, L., **Mészáros, B.**, Bajer, K., Török, J., Martín, J., Molnár, O. Oxidatív stressz: hőmérséklet hatása a nászszínezetre eltérő élőhelyű gyíkfajoknál. poster: *11. Magyar Ökológus Kongresszus* (28 – 30 August 2018, Nyíregyháza, Hungary)

Molnár, O., **Mészáros, B.**, Bajer, K., Jordán, L., Török, J., Martín, J. A Hamilton-Zuk hipotézis interspecifikus predikciójának tesztelése Nyakörvesgyíkféléken. presentation: *11. Magyar Ökológus Kongresszus* (28 – 30 August 2018, Nyíregyháza, Hungary)

Mészáros, B., Herczeg, G., Bajer, K., Török, J., Molnár, O. Environmental stress on physiological state and sexual signals of male European green lizards. előadás: *Student Conference on Conservation Science* (2 September – 29 August 2017, Tihany, Hungary)

Mészáros, B., Herczeg, G., Bajer, K., Török, J., Molnár, O. Stressz, fiziológia és szexuális jelzések: miként hat a változó környezet zöld gyíkok (*Lacerta viridis*) egészségi

állapotára és szignáljaira? presentation: *V. Herpetológiai Előadóülés* (7 March 2016, Budapest, Hungary)

Mészáros, B., Herczeg, G., Bajer, K., Török, J., Molnár, O. Környezeti stressz hatása a szexuális szignálra és egészségi állapotra: kísérletes vizsgálat zöld gyíkon (*Lacerta viridis*). poster: *Magyar Etológiai Társaság XVII. Konferenciája* (27 – 29 November 2015, Dobogókő, Hungary)

8. Other publications and conferences

Horváth, G., **Mészáros, B.**, Urszán, T. J., Bajer, K., Molnár, O., Garamszegi, L. Zs., Herczeg, G. (2017) Environment-dependence of behavioural consistency in adult male European green lizards (*Lacerta viridis*). *PLoS ONE* 12(11): e0187657. https://doi.org/10.1371/journal.pone.0187657 (IF: 3,000)

Molnár, O., Bajer, K., **Mészáros, B.**, Török, J., Herczeg, G. (2013) Negative correlation between nuptial throat colour and blood parasite load in male European green lizards supports the Hamilton–Zuk hypothesis. *Naturwissenschaften*. 100: 551. https://doi.org/10.1007/s00114-013-1051-4 (IF: 2,229)

Mészáros, B., Bajer, K., Török, J., Molnár, O., Herczeg, G. (2014) Vérparazita fertőzés és minőségjelző bélyegek kapcsolata a zöld gyíknál (*Lacerta viridis*). pp. 33-43., 11 p. In: Vágvölgyi, Cs.; Szekeres, A. (eds.) A biológia jövője, a jövő biológusai: avagy szemelvények a magyarországi felsőoktatási intézményekben végzett tudományos munka eredményeiből. Válogatás a XXXI. Országos Tudományos Diákköri Konferencia Biológia szekciójának dolgozataiból. *JATEPress Kiadó, Szeged*, Hungary.

Mészáros, B., Bajer, K., Török, J., Molnár, O., Herczeg, G. Vérparazita fertőzés és minőségjelző bélyegek kapcsolata a zöld gyíknál (*Lacerta viridis*). presentation: *Tavaszi Szél Konferencia* (21 – 23 March 2014, Debrecen, Hungary)

Mészáros, B., Bajer, K., Török, J., Molnár, O., Herczeg, G. Blood parasite load and individual quality in male European Green Lizard (*Lacerta viridis*). poster: *17th European Congress of Herpetology* (22 – 27 August 2013, Veszprém, Hungary)

Molnár, O., Bajer, K., **Mészáros, B.**, Török, J., Herczeg, G. Blood parasite load and sexual colouration in the European Green Lizard (*Lacerta viridis*). presentation: *Symposium on the ecology, evolution, and behavior of parasites and pathogens* (30 November 2012, Budapest, Hungary)

9. References

Andersson, M. (1994). Sexual selection. Princeton, Princeton University Press.

Angilletta, M.J., Niewiarowski, P.H. és Navas, C.A. (2002) The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* 27(4): 249-268. DOI: 10.1016/s0306-4565(01)00094-8.

Bajer, K., Molnár, O., Török, J. és Herczeg, G. (2010) Female European green lizards (*Lacerta viridis*) prefer males with high ultraviolet throat reflectance. *Behavioral Ecology and Sociobiology* 64(12): 2007-2014. DOI: 10.1007/s00265-010-1012-2.

Bajer, K., Molnár, O., Török, J. és Herczeg, G. (2011) Ultraviolet nuptial colour determines fight success in male European green lizards (*Lacerta viridis*). *Biology Letters* 7(6): 866-868. DOI: 10.1098/rsbl.2011.0520.

Bajer, K., Molnár, O., Török, J. és Herczeg, G. (2012) Temperature, but not available energy, affects the expression of a sexually selected ultraviolet (UV) colour trait in male European green lizards. *Plos One* 7(3): 7. DOI: 10.1371/journal.pone.0034359.

Cooper, J.E. (2001) Parasites and birds: the need for fresh thinking, new protocols and co-ordinated research in Africa. *Ostrich*: 229-232.

Darwin, C. (1871). The descent of man and selection in relation to sex. London, Murray.

Fang, Y.Z., Yang, S. és Wu, G.Y. (2002) Free radicals, antioxidants, and nutrition. *Nutrition* 18(10): 872-879. DOI: 10.1016/s0899-9007(02)00916-4.

Hamilton, D.G., Whiting, M.J. és Pryke, S.R. (2013) Fiery frills: carotenoid-based coloration predicts contest success in frillneck lizards. *Behavioral Ecology* 24(5): 1138-1149. DOI: 10.1093/beheco/art041.

Hamilton, W.D. és Zuk, M. (1982) Heritable true fitness and bright birds: a role for parasites? *Science* 218(4570): 384-387. DOI: 10.1126/science.7123238.

Huey, R.B. és Kingsolver, J.G. (1989) Evolution of thermal sensitiviy of ectotherm performance. *Trends in Ecology & Evolution* 4(5): 131-135. DOI: 10.1016/0169-5347(89)90211-5.

Ibanez, A., Polo-Cavia, N., Lopez, P. és Martin, J. (2014) Honest sexual signaling in turtles: experimental evidence of a trade-off between immune response and coloration in red-eared sliders *Trachemys scripta elegans*. *Naturwissenschaften* 101(10): 803-811. DOI: 10.1007/s00114-014-1219-6.

Lefcort, H. és Blaustein, A.R. (1991) Parasite load and brightness in lizards-an interspecific test of the Hamilton and Zuk hypothesis. *Journal of Zoology* 224: 491-499. DOI: 10.1111/j.1469-7998.1991.tb06038.x.

Martín, J. és López, P. (2009) Multiple color signals may reveal multiple messages in male Schreiber's green lizards, *Lacerta schreiberi*. *Behavioral Ecology and Sociobiology* 63(12): 1743-1755. DOI: 10.1007/s00265-009-0794-6.

Metcalfe, N.B. és Alonso-Alvarez, C. (2010) Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology* 24(5): 984-996. DOI: 10.1111/j.1365-2435.2010.01750.x.

Names, G., Martin, M., Badiane, A. és Le Galliard, J.F. (2019) The relative importance of body size and UV coloration in influencing male-male competition in a lacertid lizard. *Behavioral Ecology and Sociobiology* 73(7): 14. DOI: 10.1007/s00265-019-2710-z.

Pike, T.W., Blount, J.D., Bjerkeng, B., Lindstrom, J. és Metcalfe, N.B. (2007) Carotenoids, oxidative stress and female mating preference for longer lived males. *Proceedings of the Royal Society B-Biological Sciences* 274(1618): 1591-1596. DOI:

10.1098/rspb.2007.0317.
Polo-Cavia, N., Lopez, P. és Martin, J. (2013) Head coloration reflects health state in the red-eared slider *Trachemys scripta elegans*. *Behavioral Ecology and Sociobiology* 67(1): 153-162. DOI: 10.1007/s00265-012-1435-z.
Schall, J.J. és Staats, C.M. (1997) Parasites and the evolution of extravagant male characters: Anolis lizards on Caribbean islands as a test of the Hamilton-Zuk hypothesis. *Oecologia* 111(4): 543-548. DOI: 10.1007/s004420050269.